Disorders of Heme Synthesis

Introduction

In the last lesson (Heme/Onc: Anemia #1: *Hemoglobin*), we gave you a truncated version of the heme synthesis pathway. We encourage you to continue that perspective. Though we will again show you the complete pathway, we actively discourage you from attempting to memorize it. You do not need to know the pathway, only the enzyme deficiencies and the substrate that accumulates as a result of that deficiency. Recognize the syndrome, know the enzyme and the substrate. The best part? In the cytoplasm portion of heme synthesis, the enzyme defect and the substrate that accumulates share the same name; you need not memorize the entire pathway. Instead, learn only the name of the enzyme defect. In the mitochondrial portion of heme synthesis, you need only know d-ALA synthetase and ferrochelatase.

This distinction between mitochondrial portion and cytoplasmic portion may seem arbitrary at first. We've also omitted some diseases that can occur along this pathway, so the distinction may also seem trivial. But what we're doing we're doing deliberately to facilitate your understanding of these conditions. What we want you to see is that **mitochondrial disorders produce anemia** (sideroblastic anemia and lead poisoning) because of deficiency in heme synthesis, while **cytoplasmic disorders produce porphyria** (acute intermittent porphyria and porphyria cutanea tarda) because of accumulation of incomplete porphyrin rings. The diseases of the mitochondria stay in the mitochondria and are disorders of red blood cell maturation (mitochondria are ejected from erythroblasts before erythrocyte maturation). The diseases of the cytoplasm spill into other tissues and are disorders of other tissues.

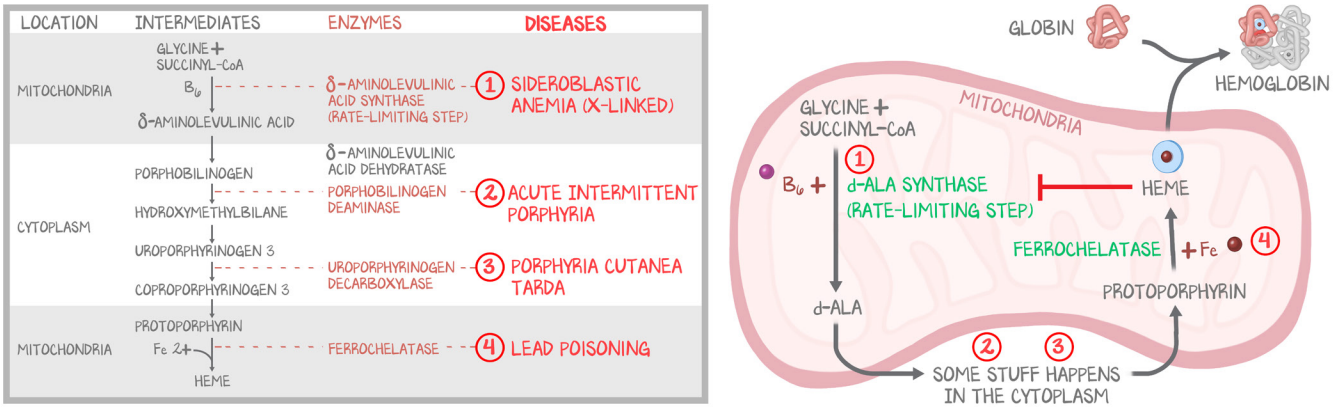


Figure 2.1: The Heme Synthesis Pathway
The real deal is on the left. Do not learn this. It is given for your reference as you read this lesson. The OME approach to this issue is on the right. In the mitochondria, you must memorize the enzymes, substrates, and products. In the cytoplasm, learn only acute intermittent porphyria and porphyria cutanea tarda, as their enzyme defect matches the name of the substrate that accumulates.

δ-Aminolevulinic acid will henceforth be referred to only as d-ALA.

Sideroblastic Anemia

Sideroblastic anemia is the anemia characterized by **ringed sideroblasts** on **bone marrow biopsy**.

Erythrocyte precursors in the bone marrow undergo several divisions before terminally differentiating into erythrocytes. Those precursors have mitochondria and a nucleus. "Ringed sideroblasts" describes the early erythroid precursors ("*-blasts*") that are full of iron-laden ("*sidero-*") mitochondria. Those mitochondria happen to form on the nucleus, which is round, forming a ring of iron-laden

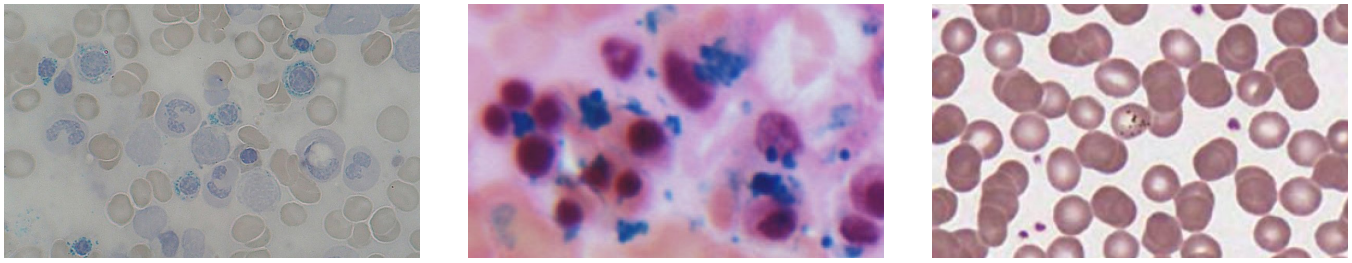
mitochondria around the nucleus ("*ringed*"). The iron is within the mitochondria because iron is delivered directly to the mitochondria via endocytosis of iron-rich ferritin and fusion of the ferritin-filled-vesicles directly into the mitochondria (Heme/Onc: Anemia #3: *Iron Regulation*). In sideroblastic anemia, heme has trouble forming. With reduced heme synthesis, there is also a reduced iron consumption. Since the mechanism to deliver iron (ferritin) is not disrupted but the use of that iron is disrupted, iron accumulates within mitochondria.

The **congenital form** of sideroblastic anemia is the result of an **x-linked recessive d-ALA synthetase deficiency**. Sideroblastic anemia is caused by a defect in the rate-limiting step of heme synthesis, which is also the first. Because less succinyl-CoA and glycine are used to make d-ALA, and there is no other entry point into this synthesis pathway, **none of the intermediates can accumulate.** The heme synthesis pathway is regulated by the presence of heme or glucose, which inhibit d-ALA synthetase. There is no feedforward signal. With a deficiency of d-ALA synthetase, the heme synthesis process doesn't get started. But the signal to 'bring more iron to marrow' comes from outside this system, from hepcidin and erythropoietin, so iron is still delivered to the mitochondria of erythroid precursors. The combination of less heme synthesis (less iron used, or less iron out) and normal iron input (no change to iron in) results in **accumulation of iron in mitochondria.** Because there is SOME d-ALA synthetase working, there is one thing we can do to facilitate more heme synthesis—**vitamin B_6, pyridoxine**. B_6 is a cofactor for d-ALA synthetase. The congenital form tends to respond well to B_6 administration, as all the endocrine signals are working well, and the erythroid precursors just need a little push to start making more heme.

There are several **acquired forms** of sideroblastic anemia. One acquired form comes in the way of a **B_6 deficiency**, almost always tested against the antimycobacterial drug **isoniazid**. In fact, we at OME teach "*isoniazid, don't forget your B_6*" as the name of the drug most other people call "*isoniazid*." In this particular cause of acquired sideroblastic anemia, because isoniazid induces a B_6 deficiency which induces the sideroblastic anemia, it does respond well to B_6. **The other acquired causes generally do not.** They include lead poisoning, copper deficiency, chronic alcohol, and myelodysplastic syndrome.

The patient will have the symptoms of anemia (Heme/Onc: Anemia #4: *Approach to Anemia*) and a low hemoglobin. A blood smear might show you basophilic stippling, but that is a trap. We want you learning that basophilic stippling is a product of lead poisoning, not sideroblastic anemia. A patient with lead poisoning, and therefore basophilic stippling, might also have lead-induced sideroblastic anemia. The diagnosis of sideroblastic anemia can only be made definitively by a **bone marrow biopsy** that demonstrates **ringed sideroblasts**. Ringed sideroblasts are not found in blood and cannot be seen on a blood smear.

In order to stain iron, we use **Prussian blue** stain, which stains iron **blue**.



(a) (b) (c)

Figure 2.2: Lead Poisoning
(a) Ringed sideroblasts on bone marrow biopsy confirm sideroblastic anemia. (b) Iron stains blue with Prussian blue, revealing mitochondria forming a ring around nuclei. Lead poisoning can cause sideroblastic anemia. Lead poisoning can also cause basophilic stippling. (c) Basophilic stippling can be seen on a peripheral blood smear, dark blue dots within red blood cells.

Lead Poisoning

Lead poisoning used to be a major concern for the development of children. Through public health efforts and public awareness, contamination of children by lead has significantly decreased. Children are exposed to lead in the forms of **house paint before 1978**, pottery glaze, and contamination of the water supply. Lead in the ground can get into the water supply. Children also seem to absorb more ingested lead (50%, compared to adults at 15%). And since lead goes wherever calcium goes, if children eat lead, children are at greater risk for developing bone disorders. Lead poisoning is more common in children, but the severity of lead poisoning in children is usually less severe than in adults.

Adults contact lead through occupational exposure. Accidental exposure can occur in patients who **shoot guns** (especially those who run gun ranges or reload their own ammunition, exposing themselves to inhaled lead and powder lead). Other occupational exposures occur in the manufacturing of batteries, car radiators, and tin cans. Lead poisoning is less common in adults, though the levels tend to get higher and the cases more severe.

Lead interferes with the normal remodeling of cartilage and primary bone trabeculae in the epiphyses of children. Lead gets into the epiphysis instead of calcium, leading to increased bone density detected as radiodense "lead lines" on X-ray. Another type of "lead line" appears in the gums as a result of hyperpigmentation. These lead lines carry the eponym Bruton's lines, and are deep blue pigmentation where the gums meet the teeth. Lead inhibits the healing of fractures by increasing chondrogenesis and delaying cartilage mineralization. Lead acting as calcium in bone explains how lead poisoning contributes to **growth retardation**.

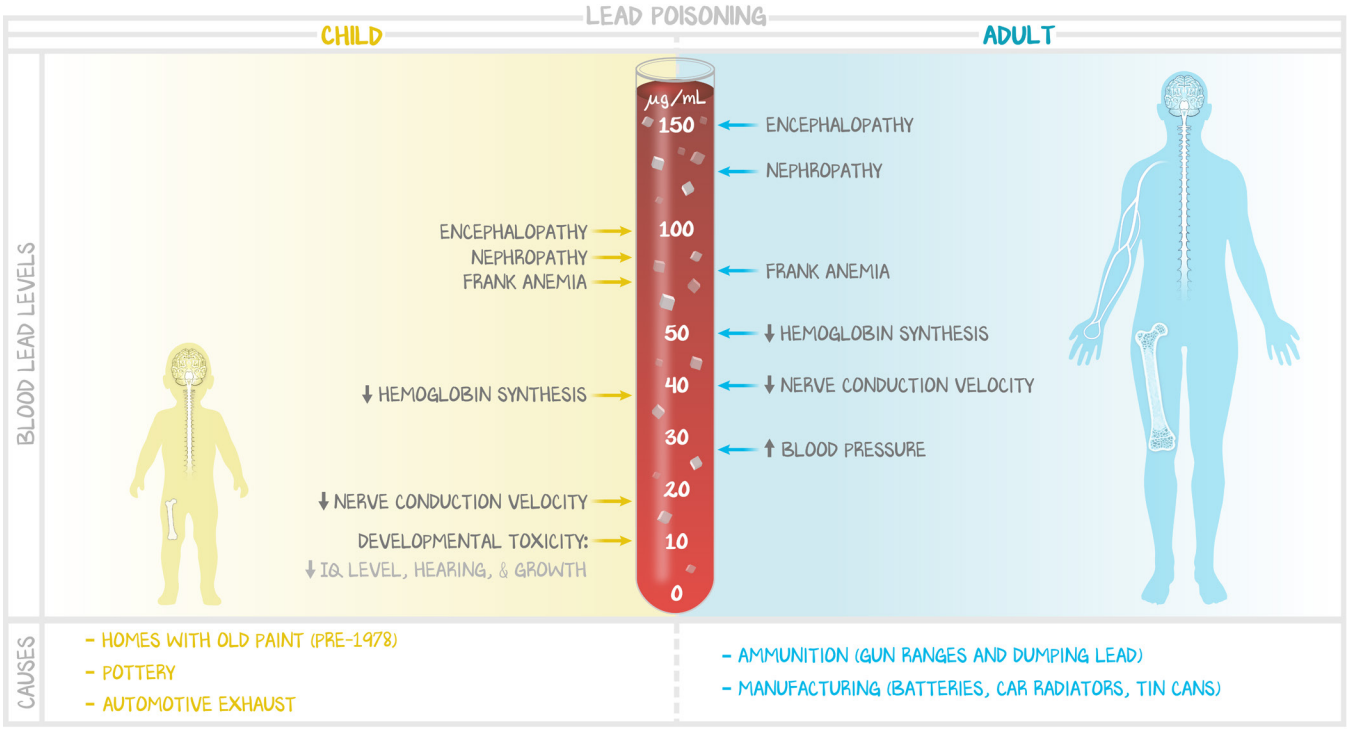
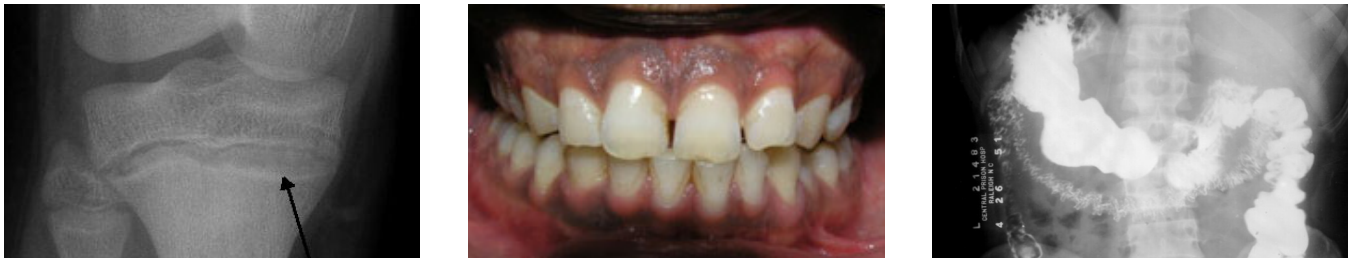


Figure 2.3: Lead Poisoning
We are learning lead in the context of anemia because it causes sideroblastic anemia. Lead does more than just cause anemia, however. Children develop symptoms—nerve conduction, anemia, nephropathy, and encephalopathy at much lower levels than adults. The more lead, the more severe the disease. Children more readily absorb lead and suffer at lower lead levels. Most critical to pediatrics is that even minute levels of lead can lead to developmental toxicity.

Lead also messes with the brain. In children more than adults, it produces encephalopathy, likely from damage to a maturing brain's vulnerable epithelium. This epithelial damage can be evidenced by cerebral edema and papilledema. As indicated by the previous figure, encephalopathy and renal failure do not occur until quite high levels. However, the subclinical impact on developing children may not be so apparent. Even low levels of lead poisoning have been linked with **mental retardation**.



(a) (b) (c)

Figure 2.4: Lead Poisoning Consequences
(a) A radiograph demonstrating lead lines at the epiphyseal plate. Note the large gap of bone (all the dark black) indicating this is a pediatric film. (b) Lead lines appear on the gums, a blue hue where the teeth arise from the gingiva. (c) Double contrast barium enema demonstrates a featureless descending and sigmoid colon, lacking normal haustral markings.

In those adults who are exposed to lead, who are at risk of acquiring lead poisoning, the levels of lead can get quite high. Lead takes the place of calcium, and without lead chelation therapy, stays in the human body for 30 years. The signs and symptoms of lead poisoning do not become abundantly clear until lead levels reach 45, the first symptom being anemia. **Peripheral neuropathy** is seen more often in adults than in children, and is a product of defective myelin synthesis. It affects the **motor neurons** of the more commonly used muscles (calcium influx induces vesicle fusion in the presynaptic neuron; lead goes where calcium goes), resulting classically in **wrist drop** and **foot drop**. As lead levels increase, more organ systems are compromised. **Lead colic** (abdominal pain caused by lead deposition in the GI tract) and **renal failure** occur as lead levels approach 100. In the triple digits, frank **encephalopathy** precedes **death** at lead levels around 150.

The treatment of lead poisoning involves **eliminating the lead exposure**, first and foremost. **Lead encephalopathy** is a medical emergency, requires intensive care monitoring, and is always treated with **intravenous EDTA** and parenteral **dimercaprol**. Intravenous therapy can only be continued for five days, at which time oral therapy is resumed with **succimer** (DMSA). The CDC states that anyone with a lead level of 45 or higher needs to be treated. Symptomatic patients are treated, obviously. But what to do about people below 45 is a bit of a controversy. Several physician organizations recognize that any lead level is deleterious, a position those of us at OME agree with. The EPA and OSHA have requested that the government impose mandatory withdrawal of a person working in a lead-exposure occupation, should any one lead level be > 30, or any two within four weeks be > 20. Fortunately, since imposing lead laws on paint and bringing awareness to the community, those previously at greatest risk (children) have shown an average decrease from 15 to 2. The point is that government policy and public awareness have hugely reduced the risk of physical and mental stunting that comes from lead poisoning, and today those who need treatment are adults who expose themselves voluntarily. The people you will treat will be adults with very high lead levels.

If heme synthesis is impaired, and lead anemia diagnosed, the patient meets criteria for treatment with **succimer** (DMSA). Our emphasis returns to the mechanism of disease as it relates to heme synthesis.

Lead inhibits the activity of two enzymes involved in heme synthesis, δ-aminolevulinic acid dehydratase (**d-ALA dehydratase**) and **ferrochelatase**. Blocking d-ALA dehydratase causes an accumulation of d-ALA (this is a cytoplasmic enzyme, and as we've said, cytoplasmic enzymes are named by the substrate that accumulates). Blocking ferrochelatase does cause an accumulation of protoporphyrin (it is in the mitochondria, so memorize it), but more importantly it causes an accumulation of iron. Just as in d-ALA synthetase deficiency, where heme synthesis was impaired but iron signaling not impaired, by blocking the final step in the addition of iron to protoporphyrin, heme synthesis is decreased, **and iron accumulates**. Therefore, lead poisoning is one cause of **sideroblastic anemia**. Lead denatures proteins involved in heme synthesis—d-ALA dehydratase and ferrochelatase—but also denatures proteins involved in making an immature RBC look like a mature RBC—ribonuclease. With inhibition of ribonuclease, the ribosomes of the erythroid precursor persist in the mature RBC. The presence of ribosomes in the cytoplasm of mature RBCs is called **basophilic stippling**. This is why we want you associating basophilic stippling with lead poisoning specifically, and not all sideroblastic anemias.

Figure 2.2, above, shows examples of basophilic stippling. Sideroblastic anemia caused by lead poisoning may present with basophilic stippling. Not all sideroblastic anemias are caused by lead poisoning, and not all sideroblastic anemias present with basophilic stippling. Learn that lead poisoning causes basophilic stippling and also causes sideroblastic anemia.

Porphyrias

Porphyrias in general are extremely rare. In all of Robbins and Cotran's "Big Robbins," they got a paragraph. All of them got ONE paragraph. We recognize and teach two as being commonly tested. The key in both diseases is that there is a defective **cytoplasmic enzyme** that results in **accumulation of substrate**, the molecule that the enzyme acts upon. Both the enzyme and the substrate share the same name. The accumulation of precursor **does not result in anemia** but instead in **deposition in other tissue**. Deposition in other tissue means the skin in porphyria cutanea tarda (cutaneous = skin), and the brain, urine, and GI tract in acute intermittent porphyria.

These diseases are caused by accumulation of intermediate substrates. Because the patients are alive (they have hemoglobin), the enzymes cannot be missing, only deficient. When the demands of the system increase, when more heme is asked to be made, the deficient enzyme cannot work harder. The preceding enzymes can, however. Which means in times of increased heme synthesis, these diseases exacerbate. More precursor is made, the deficiency enzyme cannot increase its activity, so the precursor accumulates. Since the only regulation on this system is negative inhibition by **glucose** or **heme** itself, **precipitants can only be things that reduce heme or glucose**. By increasing the elimination of heme, the pathway is disinhibited, and substrate accumulates. Increasing elimination of heme occurs from **P450 induction** (chronic EtOH, OCPs). If ever a patient becomes hypoglycemic, there can be an acute disinhibition of the system, so **fasting** can precipitate symptoms. Likewise, if there is an acute attack, **intravenous glucose** can abort the attack. Next we cover the specifics of two porphyrias.

Acute Intermittent Porphyria (AIP)

AIP is caused by **porphobilinogen deaminase deficiency**, which leads to an accumulation of porphobilinogen. That's the porphyrin ring (porpho-) and a thing that looks like the breakdown product of heme, bilirubin's (-bilin-) precursor (-ogen). It is an **autosomal dominant** inheritable disorder. The disease is called acute intermittent, which means the symptoms are only around intermittently and are sudden-onset, but go away quickly. This porphyria, more than the others, is **precipitated** by fasting or increased heme clearance.

The acute increase in attempted heme synthesis increases the concentration of porphobilinogen, which falls out of the marrow and accumulates in all tissues, but especially affects the brain and GI tract. The "classic" pattern of the disease can be recalled by the 5 P's—Pain, Polyneuropathy, Psychiatric, Port-wine urine, and Precipitants. **P**ain refers to gastrointestinal distress. The pain is so bad that the condition mirrors the acute abdomen, and patients may have been operated on for acute porphyria attacks. **P**olyneuropathy refers to the weakness and loss of sensory findings that can accompany attacks, and is categorized, along with the **P**sychiatric complaints (altered mental status, psychosis), under "problems with the nervous system." **P**ort-wine-colored urine refers to the **windowsill test**. Urine is initially voided clear, but when placed in the ultraviolet rays of the sun, the porphobilinogen oxidizes to porphobilin, giving the urine a deep red color. **P**recipitants are OCPs, chronic alcohol, and fasting.

Immediate treatment involves **intravenous glucose**. Glucose inhibits d-ALA synthetase, reducing the input into the heme synthesis pathway. Eventually, the defective-but-not-absent porphobilinogen deaminase will catch up and clear the excess porphobilinogen, and the attack will stop. Long-term treatment means avoiding precipitants—anything that induces the P450 system.

Porphyria Cutanea Tarda (PCT)

PCT is caused by a **uroporphyrinogen decarboxylase deficiency** (UROD), which leads to an accumulation of **uroporphyrinogen**: found in urine (*uro-*), and is a porphyrin ring (*-porphyrin-*) precursor molecule (*-ogen*). PCT can be provoked by the administration of precipitants, but should be learned as NOT acute and NOT intermittent. PCT is a disease that is always active. Uroporphyrinogen accumulates in the urine and the skin. It is present all the time in the skin and urine. The main presentation of the disease is a **blistering of sun-exposed areas**, a type of photosensitivity. The UV light oxidizes the uroporphyrinogen to uroporphyrin. **Uroporphyrin 1 in urine** and photosensitivity is sufficient to make the diagnosis.

Type 1 PCT is by far the most common, and has a **genetically normal UROD**. Precipitants such as alcohol or OCPs can exacerbate the condition, but the interesting finding is that nearly all of type 1 PCT is associated with **hepatitis C infection**. People with HCV can suffer from PCT, and the causal link is unclear. In type 2 PCT, there is reduced UROD activity. Unlike the windowsill test of AIP, the urine of PCT **is dark red on voiding**, and glows a **coral red under Wood's lamp.** Treatment is to avoid attacks.